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# Invasive and Transcranial Photoacoustic Imaging of the Vascular Response to Brain Electrical Stimulation

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## ABSTRACT

Advances in the brain functional imaging greatly facilitated the understanding of neurovascular coupling. For monitoring of the microvascular response to the brain electrical stimulation *in vivo* we used optical-resolution photoacoustic microscopy (OR-PAM) through the cranial openings as well as transcranially. Both types of the vascular response, vasoconstriction and vasodilatation, were clearly observed with good spatial and temporal resolution. Obtained results confirm one of the primary points of the neurovascular coupling theory that blood vessels could present vasoconstriction or vasodilatation in response to electrical stimulation, depending on the balance between inhibition and excitation of the different parts of the elements of the neurovascular coupling system.

**Keywords:** brain imaging, vasoconstriction, vasodilatation, brain electrical stimulation, optical-resolution photoacoustic microscopy

## INTRODUCTION

The question concerning localization of functions is one of the main concerns in neuroscience, and a number of new imaging techniques have enabled scientists to visualize the functional maps in the brain directly. The processing of sensory information and decision making is carried out by millions of cells, forming neural networks. In the mammalian brain, neurons which perform a given function, or share common functional properties, are grouped together with other neurons, glial cells and capillary beds. Attaining an understanding of the three-dimensional functional organization of a particular part of the neocortex is one of the most important steps towards revealing the mechanisms of cellular interactions there.

Photoacoustic imaging reflects hemodynamic activities rather than brain cell metabolism [5, 6]. Since hemodynamics indirectly reflect neural activities evoked by a particular stimulus, the visualization of functional maps is possible based on these activities in the neocortex. Therefore, we can reasonably assume that the photoacoustic imaging can also be applied to the visualization of the functional status of the cortical parenchyma including small blood vessels.

The experimental setup for laser-induced photoacoustic imaging was developed to image animal brain structures noninvasively with the skull intact as well as through hole in the bone. Our imaging modality combines the advantages of optical contrast and optical or ultrasonic resolution in the relatively large (few square mm) area of recording to observe changes in brain vasculature with good spatial and temporal resolution. Despite the fact that spatial resolution is limited by the received photoacoustic waves, in the case of transcranial imaging, it is still comparable with imaging of the open brain.

## METHODS

Before each experiment, a Swiss Webster mouse (Hsd:ND4, 25–30 g; Harlan, Indianapolis, IN) was anesthetized by intraperitoneally administering a dose of 80 mg/kg ketamine and 10 mg/kg xylazine [8]. Hair was removed from the

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head by an electric shaver and then the animal was mounted on a custom stereotaxic imaging stage, where its scalp was surgically removed. In transdural experiments, a cranial opening ( $\sim 4 \text{ mm} \times 4 \text{ mm}$ ) was made using a dental drill, and the exposed dura mater surface was cleaned with artificial cerebrospinal fluid. Throughout the experiment, the animal was kept anesthetized using vaporized 1.0% isoflurane with an air flow rate of 1.0 L/min. The body temperature of the animal was maintained at  $37^\circ\text{C}$  by a temperature-controlled heating pad. A monopolar tungsten electrode (impedance:  $1 \text{ M}\Omega$ ; tip diameter:  $10 \text{ }\mu\text{m}$ ) was introduced into the cortex to a depth of  $\sim 0.1\text{--}0.2 \text{ mm}$  through the openings [9].

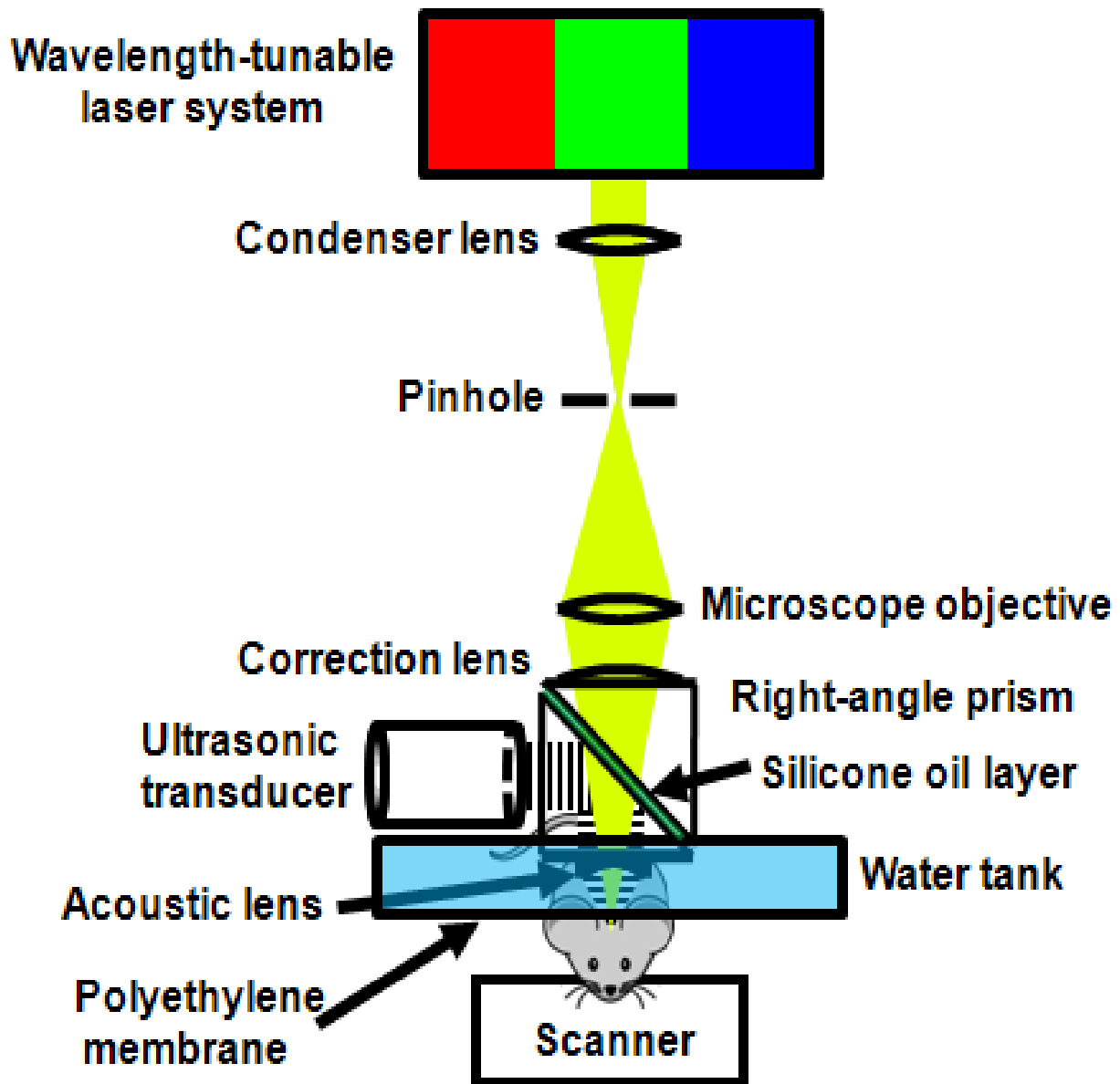


Figure 1. Schematic of optical resolution photoacoustic microscopy system. It employs optical focusing to achieve micrometer-level lateral resolution. A dye laser pumped by a laser is used as the irradiation source. Laser pulses from the dye laser are spatially filtered by a  $25 \text{ }\mu\text{m}$  diameter pinhole. The optical objective lens and  $75 \text{ MHz}$  ultrasonic transducer are coaxially and confocally configured.

In transcranial imaging, the electrode was introduced through a small hole made by 0.3 mm dental drill. Ultrasonic gel was applied to the exposed cortical surface to match the acoustic impedance [8]. After the experiment, the animal was sacrificed by intraperitoneal injection of 2.0 ml Sleepaway.

Real-time monitoring of the vascular response caused by electrical stimulation through the microelectrode was performed by repetitive line-scanning at 570 nm near the tip of the electrode. Each experiment consisted of 1–7 trials with stimulation currents 100 – 300  $\mu$ A, and each trial consisted of 300 line-scans. The time interval between each sequential line-scan was  $\sim$ 1 s. Two electrical stimulations were executed during one trial, starting at time points 100 s and 200 s, after the first laser excitation, respectively. Each stimulus consisted of a four 0.3 ms pulses at 300 Hz, generated by a stimulator (A365; World Precious Instrument, Sarasota, FL) triggered by a function generator (DS345; Stanford Research Systems, Sunnyvale, CA).

Experimental data was processed offline after the experiment. Based on the structural information, vessels crossed by the scanning line were chosen for response study. The cross-section of each vessel was first identified by image processing, and signal amplitudes were extracted through Hilbert transform [5, 7]. The peak signal amplitudes were added up across the vessel. If we assume that the packed cell volume is constant within the main vasculature, then the peak signal amplitude is proportional to the local dimension of the vessel along the optical and acoustic axis, and thus the summation of the peak amplitudes along the transverse direction across the vessel is proportional to the area of the vessel cross-section. Because each pixel of the image corresponds to a fixed physical size of  $2.5 \mu\text{m} \times 2.5 \mu\text{m} \times 7.5 \mu\text{m}$ , this method was to count the number of pixels with amplitudes above the noise level within the cross-section. The cross-section area was monitored during each trial, and normalized by the baseline which was defined as the vessel cross-sectional area before the first electrical stimulation was applied.

## RESULTS

In five open-brain experiments, vascular responses were monitored with high spatial resolution ( $\sim$ 5  $\mu\text{m}$ ) and temporal resolution of  $\sim$ 1 s. OR-PAM can image the time course of vessel response down to the capillary level. In response to the first stimulus, 61% of investigated vessels displayed no response, 16% showed vasoconstriction and 23% showed vasodilatation (n=31). In three transcranial experiments, vascular responses were monitored with spatial resolution  $\sim$ 50  $\mu\text{m}$ , but since the number of investigated vessels was limited, the percentages of responses were not calculated.

In this study we have demonstrated that our imaging technique provides not only an “open brain-type” but also a minimally invasive method for localizing regional optical properties of brain blood circulation through the skull with high ultrasonic resolution. Figure 2 shows sequential vessel cross-sectional images of typical vasodilatation in response to electrical stimulations. The maximum increase in the cross-sectional area was 150%. The time scales of vasodilatation varied, commonly, both the rising and decay times were on the order of a few tens of seconds. Figures 3 show the maximum amplitude projection images of the transcranial mouse brain microvasculature. In this experiment, the time scales of vasodilatation were consistent with open brain experiments. The data obtained transdurally and transcranially are quite similar, but transdural experiments display much better spatial resolution.

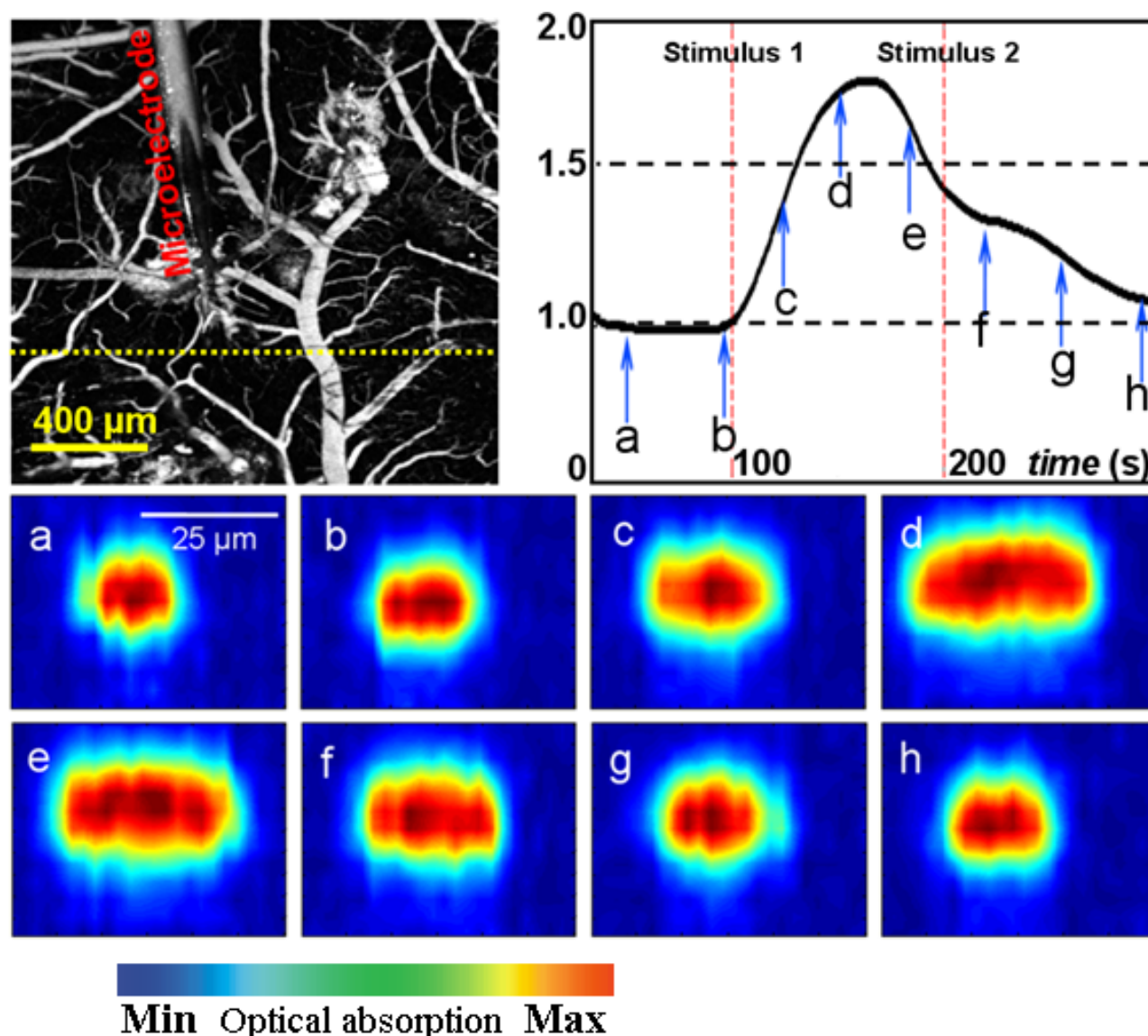


Figure 2. Top, left: photoacoustic imaging of the mouse microvasculature through the open skull. Maximum-amplitude projection (MAP) image acquired at 570 nm is shown in gray scale. A line-scan monitoring of the vascular response was performed along the dashed yellow line. Top, right: time courses of the electrical-stimulation-induced vessel cross-sectional area normalized by the baseline. Vertical axis – normalized vessel cross-section, horizontal axis – time. The two stimulations are indicated by the two vertical dashed lines, respectively. Two electrical stimulations were executed during one trial, starting at time points 100 s and 200 s, respectively. Each stimulus consisted of a train of four 0.3 ms pulses at 300 Hz. Bottom: cross-sectional images indicated by the arrows at different time points.

Since neural activity is associated with changes in oxygen consumption in the brain parenchyma [1, 2, 8]. Optical measurements during brain activation can assess hemoglobin oxygenation, and changes in the local blood circulation, including blood stream and vessel size [2, 4]. In the first part of our studies, we exposed brain tissue and photoacoustically image the brain activity has been achieved at microscopical spatial resolution. At the second part of our studies using lasers beams that can penetrate biological tissue reasonably well, it has become possible to assess brain activity in mice through the intact skull non-invasively. Advantages of the transcranial methods include a temporal resolution in the seconds range, good spatial resolution and preserving intact brain.

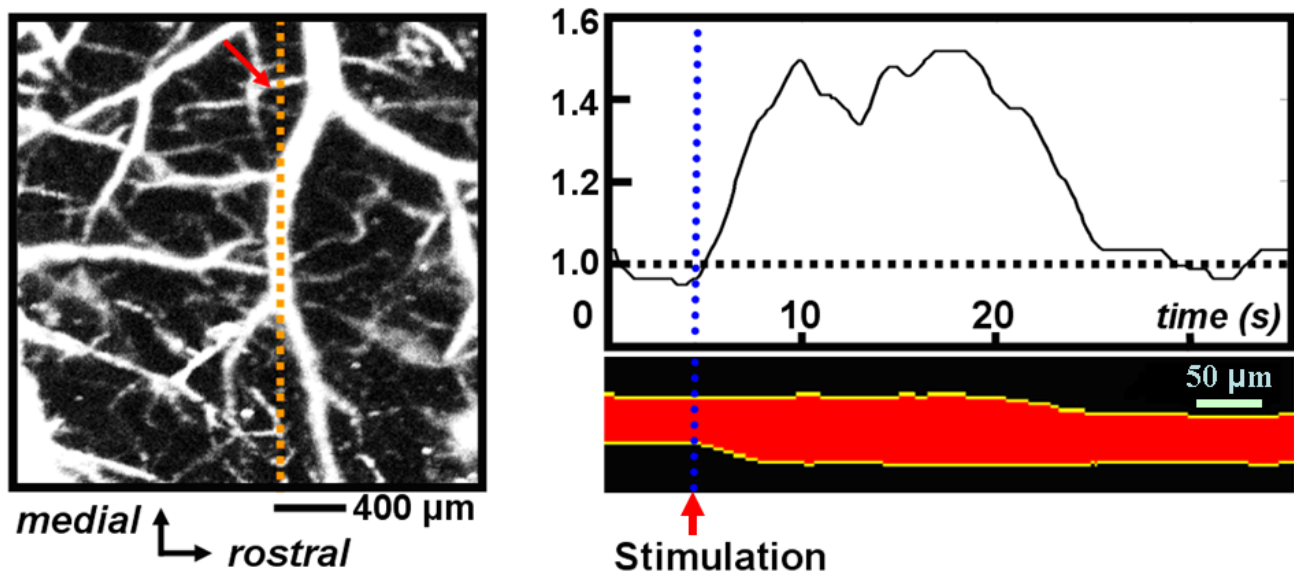


Figure 3. Top, left: transcranial photoacoustic imaging of the mouse microvasculature. Maximum-amplitude projection (MAP) image acquired at 570 nm is shown in gray scale. A line-scan monitoring of the vascular response was performed along the dashed yellow line. Top, right: time courses of the electrical-stimulation-induced vessel cross-sectional area normalized by the baseline. Vertical axis – normalized vessel cross-section, horizontal axis – time. The stimulation is indicated by the vertical dashed line. Top, right, bottom: cross-sectional images indicated by the arrows at different time points. Top, left: line-scan image of a vessel, marked by red arrow in the MAP -imaged near the tip of the microelectrode.

## DISCUSSION AND CONCLUSION

By using OR-PAM we were able to observe clearly two types of vascular responses to electrical stimulation, vasoconstriction and vasodilatation. As was shown previously [7], the neurovascular system remained at a resting level under consistent physiological conditions, which was reflected by the stable size of the vessel lumens. This balance could be deposited by local brain electrical stimulation, which could result in vasoconstriction or vasodilatation, depending on many factors: stimulus intensity, electrode location, vessel structure and type etc. [3, 8].

Vasoconstriction results from the constriction of the smooth muscles cells within blood vessel walls in response to electrical stimulation. On one hand, electrical stimulation may cause excitation of neurons resulting in synaptic release of neurotransmitters. These neurotransmitters can further react with nearby astrocytes, and endothelial cells of the vessel wall [4]. The result of these reactions drives the vessel from its resting level state to vasodilatation. Also, direct electrical stimulation on astrocytes may result in intracellular calcium waves along the astrocytic syncytium, which may signal the release of various neuromodulators from the astrocytes and neurons to the extracellular space. These neuromodulators can direct the blood vessel to regulate the metabolic supply by either increasing or decreasing the vessels lumens.

In the studies of exposed brain tissue, photoacoustic microscopy of brain activity has been achieved at high temporal and microscopical spatial resolution. Advantages of the optical methods include biochemical specificity, a temporal resolution in the seconds range, the potential of measuring intracellular and intravascular events simultaneously and the portability of the devices enabling bedside examinations, first of all capillary bed, optical measurements during brain activation can assess hemoglobin oxygenation and physical size of the single blood vessel.

In conclusion, our study has clearly demonstrated the probability of using OR-PAM to monitor the vascular response to electrical stimulation transdurally as well as transcranially. The vascular response was consistently observed at the single

vessel level with a temporal resolution of one second or even less and spatial resolution up to 10  $\mu\text{m}$ . OR-PAM is a promising tool for *in vivo* studies of the brain vasculature under a variety of experimental conditions.

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## REFERENCES

- [1] Cauli, B., et al., *Cortical GABA interneurons in neurovascular coupling: Relays for subcortical vasoactive pathways*. Journal of Neuroscience, 2004. **24**(41): p. 8940-8949.
- [2] Grinvald, A., *Cortical architecture and dynamics revealed by optical magnetic imaging*. European Journal of Neuroscience, 2000. **12**: p. 183-183.
- [3] Hu, S., et al., *Functional transcranial brain imaging by optical-resolution photoacoustic microscopy*. J Biomed Opt, 2009. **14**(4): p. 040503.
- [4] Inyushin, M.Y., A.B. Vol'nova, and D.N. Lenkov, *Use of a Simplified Method of Optical Recording to Identify Foci of Maximal Neuron Activity in the Somatosensory Cortex of White Rats*. Neuroscience and Behavioral Physiology, 2001. **31**(2): p. 201-205.
- [4] Leybaert, L., *Neurobarrier coupling in the brain: a partner of neurovascular and neurometabolic coupling?* Journal of Cerebral Blood Flow and Metabolism, 2005. **25**(1): p. 2-16.
- [5] L. Li, B. Rao, K. Maslov, and L. V. Wang, "Fast-scanning reflection-mode integrated photoacoustic and optical-coherence microscopy," Proceedings of SPIE, 7564-70 (2010).
- [6] Maslov, K., et al., *Optical-resolution photoacoustic microscopy for in vivo imaging of single capillaries*. Opt Lett, 2008. **33**(9): p. 929-31.
- [7] Metea, M.R. and E.A. Newman, *Glial cells dilate and constrict blood vessels: A mechanism of neurovascular coupling*. Journal of Neuroscience, 2006. **26**(11): p. 2862-2870.
- [8] Stein, E.W., K. Maslov, and L.H.V. Wang, *Noninvasive, in vivo imaging of blood-oxygenation dynamics within the mouse brain using photoacoustic microscopy*. Journal of Biomedical Optics, 2009. **14**(2): p. 020502.
- [9] Tsytarev, V., et al., *Imaging cortical electrical stimulation in vivo: fast intrinsic optical signal versus voltage-sensitive dyes*. Optics Letters, 2008. **33**(9): p. 1032-1034.